Heart Repair with Human Tissue Engineered Myocardium

**Reporting Period:** Year 1

Despite advances in medical and device therapies, patients with end-stage heart failure have a survival rate of only 25% during the first 2 years following their diagnosis. Heart failure typically follows from damage induced by severe myocardial infarction (MI; heart attack). After a severe MI, the human heart may lose up to 1 billion heart muscle cells (cardiomyocytes). For most of these patients, heart transplantation is the only useful therapy, but there is a severe shortage of donor hearts.

Recently, left ventricular assist devices (LVADs) have become available to take over the pumping function of the crucial left ventricle chamber of the heart. These devices were originally used as “bridge to transplant” (a temporary measure to keep patients alive until a new heart became available); recently some patients have received LVADs as “destination therapies” (permanent substitutes for transplanted hearts). The problems associated with these mechanical implants, however, include increased risk of stroke (blood clots that form due to the devices) and infection (the LVADs are powered from batteries that are carried outside the body and require wires to pierce the skin).

We are working to develop cardiac regenerative medicine using Engineered Heart Muscle (EHM). We are using human embryonic stem cells (hESCs) because they can be grown in very large quantities and, with the appropriate methods, can be triggered to differentiate into the cardiomyocytes, fibroblasts and smooth muscle that are lost after MI. Because these cells can be produced in essentially unlimited quantities, we could theoretically treat a very large number of patients who currently have no options.

During the first year of this project, we have a) established methods for producing the multi-billion quantities of hESC-derived cells needed to address this problem; b) developed methods to freeze and ship these cells to our collaborator in Germany for EHM assembly, and c) used these cells to generate 2 different forms of EHMs to compare their survival and function both in vitro (composition, force generated) and in vivo (after transplantation into rats that have been given MIs). We are now refining the EHM design with the goal of moving forward to testing them in animals with more human-like hearts (based on size and heart rate); this step will be essential to evaluate their safety and function before any clinical trial.

**Reporting Period:** Year 2

The project “Heart Repair with Human Tissue Engineered Myocardium” is designed to find a new option for the treatment of heart failure. Because of the shortage of donor hearts, many patients in need never receive this life-saving therapy. We are generating engineered heart muscles (EHMs) that are made from cardiomyocytes (heart muscle cells) derived from human embryonic stem cells. The ultimate goal of this work is to produce a beating human heart “patch” that can be transplanted onto damaged hearts, and help restore function. Through the joint efforts of researchers in Dr. Joseph Wu’s laboratory at Stanford and Dr. Larry Couture’s team at City of Hope, we have developed a process that allows essentially unlimited generation of cardiomyocytes using a process that is fully compatible with eventual clinical use. Our collaborator at Gottingen University, Dr. Wolfram Zimmerman, uses these cells to produce EHMs, which are then shipped to Stanford. At Stanford, the EHMs are evaluated for their structure, overall health, and ability to generate force as measured in vitro. These EHMs are also transplanted into rodents that have been given heart attacks, to see if the EHMs can survive and improve heart function. In the first year of this project, we compared different methods of making EHMs and the results that could be measured both in vitro and in vivo. We established a model of heart disease in rats with defective immune systems (necessary for the survival of human cells/tissues in this extremely foreign setting). We found that a specific grid-like patch design was both easier to construct than other options and was able to survive in the rat model of heart disease. In the 2nd year, we focused on this patch design and performed a larger number of transplants. Using EHMs made from genetically engineered cells that give off a fluorescent signal, we were able to track the long-term survival of the EHMs (at least 7 months) without having to sacrifice many of the animals. Our analysis of the transplanted EHMs showed that they had survived transplantation and had taken on characteristics that made them closer to normal heart tissue. In addition, EHM transplantation resulted in improved heart function, as compared to rats that either received no transplants or received a control EHM transplant that contained dead cells. The next phase of our project will be to evaluate the function of larger EHMs in swine model of heart disease, since these animals have hearts that are similar in size and heart rate to humans. This is a crucial step before considering translating this work into human patients.
Patients with end-stage heart failure (ESHF) have a particularly grave prognosis, with a 2-year survival rate of 25% despite optimized medical and device therapy. ESHF develops typically after myocardial infarction (MI). For most of these patients, orthotopic heart transplantation (OHT) is the only curative therapeutic option, but cannot be delivered to the necessary extent due to donor organ shortage- it is estimated that there are 200,000 patients diagnosed with ESHF annually, yet there are only 3,000 OHT performed per year. Thus, there is a clear unmet need for the development of alternative treatments for the growing ESHF patient population.

In ESHF patients, a left ventricular assist device (LVAD) is regularly implanted as a bridge to OHT. This device takes over the pumping role of the left ventricle and in some cases (e.g., when no suitable donor heart is available) may remain in the patient for the rest of his/her life. These LVADs represent a significant step forward in therapy, but have some associated risks: there is an increased incidence of stroke in these patients, and because the LVADs are powered by batteries residing outside the body, these patients have wires protruding through their chests that makes them susceptible to life-threatening infections. In this project, we have investigated the possibility of generating a biological LVAD: a patch composed of human embryonic stem cell-derived cardiomyocytes (heSC-CMs) that would be implanted over the damaged region of a patient’s heart. This patch (also called engineered heart muscle; EHM) could potentially provide both contractile function and also allow the cardiomyocytes in the patch to secrete factors that improve the survival and function of the host’s heart. In order to assess the potential of this new therapy, we established a collaboration between the laboratories of Dr. Joseph Wu at Stanford (an expert in understanding heart disease through the use of stem cell-derived cardiomyocytes), Dr. Wolfram Zimmerman at Göttingen (a leader in the design and generation of engineered heart muscle patches), and Dr. Larry Couture at City of Hope (a facility with the unique ability to produce billions of hESC-CMs). After transferring procedures for hESC-CM generation from the Wu lab to City of Hope, the latter developed methods to produce essentially unlimited quantities of hES-CMs. These cells were shipped to Göttingen to use in EHM generation. The subsequent EHMs were sent to Stanford for transplants into both immunodeficient rat and immunosuppressed pig models of heart failure to test the safety and efficacy of the EHMs. (Rat models are typically used to show safety of cell therapies; large animal models such as pigs are typically used because they have hearts with similar sizes and properties to humans. The large animal models are particularly challenging, because they have intact immune systems that mount strong responses against human cells and typically reject the grafts.) We were able to demonstrate long-term survival of the EHMs in immunodeficient rat models with no evidence of tumor formation and showed that the transplanted EHM helped preserve diastolic function (i.e., the function of the heart during the relaxation phase) in the damaged hearts. We also developed a new immunosuppression regimen to prolong survival of the human cell-derived EHMs in pigs, and have transplanted a series of animals with the EHMs and are monitoring effects on heart function, as well as the safety of the approach in a human-sized heart.

Heart Repair with Human Tissue Engineered Myocardium

**Grant Type:** Early Translational III

**Grant Number:** TR3-05556

**Project Objective:** Achieve a DC for heart failure to be delivered surgically. Plan includes patch generation and characterization as well as in vivo efficacy and preliminary safety work. Immune suppression regimen will be jointly developed between this award and DR2-05394.

DC to be a tissue-engineered patch of hESC-CM and mesenchymal cells (fibroblast like per team) for heart failure.

POC testing to be performed in nude rat and after immunosuppressive regimen development, in pig, 1 month post MI.
**Investigator:**

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<th>Joseph Wu</th>
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<tr>
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<tr>
<th>Name</th>
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<td>University Medical Center Goettingen</td>
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**Disease Focus:** Heart Disease

**Collaborative Funder:** Germany

**Human Stem Cell Use:** Embryonic Stem Cell

**Award Value:** $4,396,738

**Status:** Closed

**Application Title:** Heart Repair with Human Tissue Engineered Myocardium

**Public Abstract:**

Heart disease is the number one cause of morbidity and mortality in the US. With an estimated 1.5 million new or recurrent myocardial infarctions, the total economic burden on our health care system is enormous. Although conventional pharmacotherapy and surgical interventions often improve cardiac function and quality of life, many patients continue to develop refractory symptoms. Thus, the development of new therapies is urgently needed. “Tissue engineering” can be broadly defined as the application of novel bioengineering methods to understand complex structure-function relationships in normal or pathological conditions and the development of biological substitutes to restore, maintain, or improve function. It is different from “cell therapy”, which is designed to improve the function of an injured tissue by simply injecting suspensions of isolated cells into the injury site. To date, two main limitations of cell therapy are (1) acute donor cell death due to unfavorable seeding environment and (2) the lack of suitable cell type that genuinely resembles human cardiac cells. Our proposal seeks to use engineered tissue patches seeded with human embryonic stem cell-derived cardiomyocytes for treatment of ischemic heart disease in small and large animal models. It represents a significant development of novel techniques to address both of the main limitations of cell therapy, and will provide a new catalyst for the entire field of stem cell-based tissue engineering.
Patients with end-stage heart failure have a 2-year survival rate of 25% by conventional medical therapy. Not commonly known to the public is that this dismal survival rate is actually worse when compared to patients with AIDS, liver cirrhosis, or stroke. Following a heart failure, the endogenous repair process is not sufficient to compensate for cardiomyocyte death. Thus, novel therapies with stem cells in combination with supportive scaffolds to form engineered cardiac tissue grafts is emerging as a promising therapeutic avenue. Engineered tissues have now been used to make new bladders for patients needing cystoplasty, bioartificial heart patches seeded with bone marrow cells, and more recently new trachea for patient with late stage tracheal cancer. Our multi-disciplinary team intends to push the therapeutic envelop by developing human tissue engineered myocardium for treatment of post-myocardial infarction heart failure. We will first test our engineered cardiac tissue in small and large animal models. We will perform extensive quality control measures to define morphological, molecular, and functional properties. At the end of 3 years, we are confident we will be able to derive a lead candidate that can move into IND-enabling preclinical development. These discoveries will benefit the millions of patients with heart failure in California and globally.